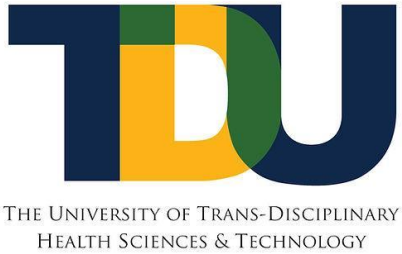

ROLE OF SCHIZOPHRENIA-ASSOCIATED TRANSCRIPTION FACTOR TCF4 IN MATURE NEURONS

SYNOPSIS

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Synopsis

Neurons perform information processing by virtue of their elaborate cellular structure, fine-tuned electrical properties and capability to integrate in various circuits. Neuronal development starts at embryonic days 9–9.5 and is completed by birth (Semple et al., 2013) although pruning of neurons continues after birth.

The dendritic arbours along with the dendritic spines are the functional units of mature neurons, enabling information processing and plasticity for cognition and memory. While the spines are more of a dynamic entity responsible for synaptic plasticity (Alvarez & Sabatini, 2007; Durand et al., 1996; Gipson & Olive, 2017), the dendritic arbour of an adult neuron is thought to be a largely stable structure (Debello & Zito, 2019; Koleske, 2013) that underlies the functional connections of a neuron within the intended circuits. Thus neurons need to actively preserve their dendritic structure for functional integrity throughout a lifespan and through situations of physiological or pathological perturbation. However, what underlies the structural integrity of mature neurons in the adult brain remains a poorly studied topic.

Mature neurons can be categorized based on their morphology, location and electrical properties. One such broad classification consists of the excitatory and inhibitory neuronal subtypes. The hippocampus, which is an indispensable region for episodic and spatial memory formation and other cognitive functions, mainly comprises excitatory neurons (Andersen et al., 2009; Graves et al., 2012). The pyramidal neurons are a class of excitatory projection neurons enriched in the CA1 and CA3 region of the hippocampus. Only recently it has come to light that CA1, the major output centre of hippocampus, shows pronounced inter-regional transcriptional heterogeneity even within the pyramidal cell type, greatest of which is along the dorso-ventral axis (Cembrowski et al., 2016). Along the long axis pyramidal cells exhibit functional differences, for example the ventral CA1 is associated with stress responses and emotional behaviour whereas the dorsal part is mostly responsible for spatial memory (R. Jones 2010). Also, the ventral pyramidal cells are more excitable than their dorsal counterparts (Cembrowski & Spruston, 2019). Single cell sequencing analysis has also discovered distinct subclasses of glutamatergic neurons in CA1 based on the enrichment of mitochondrial genes (Zeisel et al., 2015), although it's not clear whether these two classes of cells are spatially segregated along the various axes or are neighbouring cells. From existing literature, it is evident that heterogeneity among the pyramidal cells of CA1 is at least in part tied to its spatial location. But the possibility of cells sitting next to each other being distinct in their properties, function or connectivity is unexplored.

Tcf4 is a transcription factor associated with neurodevelopmental and psychiatric disorders. It is a basic helix-loop-helix protein, belonging to the E-protein family. It is continually expressed throughout the lifespan and has been proven crucial for brain development. It is expressed in the mammalian brain throughout the developmental regime and has critical role in neuronal differentiation and migration, as well as in formation of the normal brain architecture (Chen et al., 2016; Li et al., 2019; Mesman et al., 2020; Page et al., 2018; Rannals et al., 2016a). But long after the completion of neurodevelopment, Tcf4 continues to be

expressed in the adult brain, throughout the lifespan, in both neuronal and glial cell types (Jung et al., 2018; Rannals et al., 2016b; Sepp et al., 2011). But its function in a cell-type specific manner in the adult brain remained elusive. Tcf4's importance for the nervous system is evident by the fact that haplo-insufficiency of Tcf4 causes Pitt-Hopkins Syndrome (PTHS) (Amiel et al., 2007; Brockschmidt et al., 2007; Maduro et al., 2016; Zweier et al., 2007), a type of neuro-developmental disorder which manifests as intellectual disability, developmental delay, etc. Importantly, it has also been identified as a high-risk allele for Schizophrenia (Ripke et al., 2014; Stefansson et al., 2009; Steinberg et al., 2011; Williams et al., 2011) and Autism-Spectrum Disorders (O'Donnell et al., 2010; Phan et al., 2020) in large GWAS studies. Since Tcf4 is highly expressed in the adult brain throughout life and its perturbations lead to pathological conditions, we focused on investigating Tcf4's function in the adult brain neurons under normal conditions.

The first chapter of my thesis would comprise of characterization of Tcf4 expression in excitatory and inhibitory neurons in different regions of the adult brain, and identification of two populations of CA1 pyramidal neurons based on Tcf4 and CamKIIα. The second chapter would consist of Tcf4 loss-of-function analysis, using a mouse model for inducible deletion in neurons to examine its effects at cellular, structural, behavioural, electrophysiological (collaborative work) and transcriptomic level(Sarkar et al., 2021). The third chapter would constitute of some preliminary investigations of Tcf4-mediated regulation for homeostatic compensation.

References

Alvarez, V. A., & Sabatini, B. L. (2007). Anatomical and physiological plasticity of dendritic spines. *Annual Review of Neuroscience*, *30*, 79–97. https://doi.org/10.1146/ANNUREV.NEURO.30.051606.094222

Amiel, J., Rio, M., De Pontual, L., Redon, R., Malan, V., Boddaert, N., Plouin, P., Carter, N. P., Lyonnet, S., Munnich, A., & Colleaux, L. (2007). Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *American Journal of Human Genetics*. https://doi.org/10.1086/515582

Andersen, P., Morris, R., Amaral, D., Bliss, T., & O' Keefe, J. (2009). The Hippocampus Book. In *The Hippocampus Book*. https://doi.org/10.1093/acprof:oso/9780195100273.001.0001

Brockschmidt, A., Todt, U., Ryu, S., Hoischen, A., Landwehr, C., Birnbaum, S., Frenck, W., Radlwimmer, B., Lichter, P., Engels, H., Driever, W., Kubisch, C., & Weber, R. G. (2007). Severe mental retardation with breathing abnormalities (Pitt - Hopkins syndrome) is caused by haploinsufficiency of the neuronal bHLH transcription factor TCF4. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/ddm099

Cembrowski, M. S., Bachman, J. L., Wang, L., Sugino, K., Shields, B. C., & Spruston, N. (2016). Spatial Gene-Expression Gradients Underlie Prominent Heterogeneity of CA1 Pyramidal Neurons. *Neuron*, *89*(2), 351–368. https://doi.org/10.1016/j.neuron.2015.12.013

Cembrowski, M. S., & Spruston, N. (2019). Heterogeneity within classical cell types is the rule: lessons from hippocampal pyramidal neurons. *Nature Reviews Neuroscience 2019 20:4*, *20*(4), 193–204. https://doi.org/10.1038/s41583-019-0125-5

Chen, T., Wu, Q., Zhang, Y., Lu, T., Yue, W., & Zhang, D. (2016). Tcf4 controls neuronal migration of the cerebral cortex through regulation of Bmp7. *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2016.00094

Debello, W., & Zito, K. (2019). *Within a Spine ' s Reach*. http://www.sciencedirect.com:5070/book/9780128037843/the-rewiring-brain

Durand, G. M., Kovalchuk, Y., & Konnerth, A. (1996). Long-term potentiation and functional synapse induction in developing hippocampus. *Nature*, *381*(6577), 71–75. https://doi.org/10.1038/381071A0

Gipson, C. D., & Olive, M. F. (2017). Structural and functional plasticity of dendritic spines – root or result of behavior? *Genes, Brain, and Behavior*, *16*(1), 101. https://doi.org/10.1111/GBB.12324

Graves, A. R., Moore, S. J., Bloss, E. B., Mensh, B. D., Kath, W. L., & Spruston, N. (2012). Hippocampal Pyramidal Neurons Comprise Two Distinct Cell Types that Are Countermodulated by Metabotropic Receptors. *Neuron*, *76*(4), 776–789. https://doi.org/10.1016/j.neuron.2012.09.036

Jung, M., Häberle, B. M., Tschaikowsky, T., Wittmann, M. T., Balta, E. A., Stadler, V. C., Zweier, C., Dörfler, A., Gloeckner, C. J., & Lie, D. C. (2018). Analysis of the expression pattern of the schizophrenia-risk and intellectual disability gene TCF4 in the developing and adult brain suggests a role in development and plasticity of cortical and hippocampal neurons. *Molecular Autism*. https://doi.org/10.1186/s13229-018-0200-1

Koleske, A. J. (2013). Molecular mechanisms of dendrite stability. *Nature Publishing Group*. https://doi.org/10.1038/nrn3486

Li, H., Zhu, Y., Morozov, Y. M., Chen, X., Page, S. C., Rannals, M. D., Maher, B. J., & Rakic, P. (2019). Disruption of TCF4 regulatory networks leads to abnormal cortical development and mental disabilities. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0353-0

Maduro, V., Pusey, B. N., Cherukuri, P. F., Atkins, P., Du Souich, C., Rupps, R., Limbos, M., Adams, D. R., Bhatt, S. S., Eydoux, P., Links, A. E., Lehman, A., Malicdan, M. C., Mason, C. E., Morimoto, M., Mullikin, J. C., Sear, A., Van Karnebeek, C., Stankiewicz, P., … Boerkoel, C. F. (2016). Complex translocation disrupting TCF4 and altering TCF4 isoform expression segregates as mild autosomal dominant intellectual disability. *Orphanet Journal of Rare Diseases*. https://doi.org/10.1186/s13023-016-0439-6

Mesman, S., Bakker, R., & Smidt, M. P. (2020). Tcf4 is required for correct brain development during embryogenesis. *Molecular and Cellular Neuroscience*. https://doi.org/10.1016/j.mcn.2020.103502

O'Donnell, L., Soileau, B., Heard, P., Carter, E., Sebold, C., Gelfond, J., Hale, D. E., & Cody, J. D. (2010). Genetic determinants of autism in individuals with deletions of 18q. *Human Genetics*. https://doi.org/10.1007/s00439-010-0839-y

Page, S. C., Hamersky, G. R., Gallo, R. A., Rannals, M. D., Calcaterra, N. E., Campbell, M. N., Mayfield, B., Briley, A., Phan, B. N., Jaffe, A. E., & Maher, B. J. (2018). The schizophrenia-and autism-associated gene, transcription factor 4 regulates the columnar distribution of layer 2/3 prefrontal pyramidal neurons in an activity-dependent manner. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2017.37

Phan, B. D. N., Bohlen, J. F., Davis, B. A., Ye, Z., Chen, H. Y., Mayfield, B., Sripathy, S. R., Cerceo Page, S., Campbell, M. N., Smith, H. L., Gallop, D., Kim, H., Thaxton, C. L., Simon, J. M., Burke, E. E., Shin, J. H., Kennedy, A. J., Sweatt, J. D., Philpot, B. D., … Maher, B. J. (2020). A myelin-related transcriptomic profile is shared by Pitt–Hopkins syndrome models and human autism spectrum disorder. *Nature Neuroscience*. https://doi.org/10.1038/s41593-019-0578-x

Rannals, M. D. D., Hamersky, G. R. R., Page, S. C. C., Campbell, M. N. N., Briley, A., Gallo, R. A. A., Phan, B. D. N., Hyde, T. M. M., Kleinman, J. E. E., Shin, J. H. H., Jaffe, A. E. E., Weinberger, D. R. R., & Maher, B. J. J. (2016a). Psychiatric Risk Gene Transcription Factor 4 Regulates Intrinsic Excitability of Prefrontal Neurons via Repression of SCN10a and KCNQ1. *Neuron*, *90*(1), 43–55. https://doi.org/10.1016/j.neuron.2016.02.021

Rannals, M. D. D., Hamersky, G. R. R., Page, S. C. C., Campbell, M. N. N., Briley, A., Gallo, R. A. A., Phan, B. D. N., Hyde, T. M. M., Kleinman, J. E. E., Shin, J. H. H., Jaffe, A. E. E., Weinberger, D. R. R., & Maher, B. J. J. (2016b). Psychiatric Risk Gene Transcription Factor 4 Regulates Intrinsic Excitability of Prefrontal Neurons via Repression of SCN10a and KCNQ1. *Neuron*. https://doi.org/10.1016/j.neuron.2016.02.021

Ripke, S., Neale, B. M., Corvin, A., Walters, J. T. R., Farh, K. H., Holmans, P. A., Lee, P., Bulik-Sullivan, B., Collier, D. A., Huang, H., Pers, T. H., Agartz, I., Agerbo, E., Albus, M., Alexander, M., Amin, F., Bacanu, S. A., Begemann, M., Belliveau, R. A., … O'Donovan, M. C. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. https://doi.org/10.1038/nature13595

Sarkar, D., Shariq, M., Dwivedi, D., Krishnan, N., Naumann, R., Bhalla, U. S., & Ghosh, H. S. (2021). Adult brain neurons require continual expression of the schizophrenia-risk gene Tcf4 for structural and functional integrity. *Translational Psychiatry*, *11*(1), 1–11. https://doi.org/10.1038/s41398-021-01618-x

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology*, *0*, 1.

https://doi.org/10.1016/J.PNEUROBIO.2013.04.001

Sepp, M., Kannike, K., Eesmaa, A., Urb, M., & Timmusk, T. (2011). Functional diversity of human basic helix-loop-helix transcription factor TCF4 isoforms generated by alternative 5′ exon usage and splicing. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0022138

Stefansson, H., Ophoff, R. A., Steinberg, S., Andreassen, O. A., Cichon, S., Rujescu, D., Werge, T., Pietiläinen, O. P. H., Mors, O., Mortensen, P. B., Sigurdsson, E., Gustafsson, O., Nyegaard, M., Tuulio-Henriksson, A., Ingason, A., Hansen, T., Suvisaari, J., Lonnqvist, J., Paunio, T., … Myin-Germeys, I. (2009). Common variants conferring risk of schizophrenia. *Nature*. https://doi.org/10.1038/nature08186

Steinberg, S., de Jong, S., Andreassen, O. A., Werge, T., Børglum, A. D., Mors, O., Mortensen, P. B., Gustafsson, O., Costas, J., Pietiläinen, O. P. H., Demontis, D., Papiol, S., Huttenlocher, J., Mattheisen, M., Breuer, R., Vassos, E., Giegling, I., Fraser, G., Walker, N., … Stefansson, K. (2011). Common variants at VRK2 and TCF4 conferring risk of schizophrenia. *Human Molecular Genetics*, *20*(20), 4076–4081. https://doi.org/10.1093/hmg/ddr325

Williams, H. J., Craddock, N., Russo, G., Hamshere, M. L., Moskvina, V., Dwyer, S., Smith, R. L., Green, E., Grozeva, D., Holmans, P., Owen, M. J., & O'Donovan, M. C. (2011). Most genome-wide significant susceptibility loci for schizophrenia and bipolar disorder reported to date cross-traditional diagnostic boundaries. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/ddq471

Zeisel, a., Manchado, a. B. M., Codeluppi, S., Lonnerberg, P., La Manno, G., Jureus, a., Marques, S., Munguba, H., He, L., Betsholtz, C., Rolny, C., Castelo-Branco, G., Hjerling-Leffler, J., & Linnarsson, S. (2015). Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science*, *347*(6226), 1138–1142. https://doi.org/10.1126/science.aaa1934

Zweier, C., Peippo, M. M., Hoyer, J., Sousa, S., Bottani, A., Clayton-Smith, J., Reardon, W., Saraiva, J., Cabral, A., Göhring, I., Devriendt, K., De Ravel, T., Bijlsma, E. K., Hennekam, R. C. M., Orrico, A., Cohen, M., Dreweke, A., Reis, A., Nurnberg, P., & Rauch, A. (2007). Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). *American Journal of Human Genetics*. https://doi.org/10.1086/515583